Investigating Muscle Repair by Inducing Cold Damage in *Drosophila melanogaster*

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Introduction

Insects frequently encounter sudden cold events that can disrupt ion balance, depolarize muscle membranes, and damage contractile structures. While vertebrate muscle repair is well charactarized, whether insects can repair freezing-induced muscle injury remains unclear. *Drosophila melanogaster* provides an ideal system for testing this because cold hardening (CH) enhances survival long enough to examine muscle-level consequences of freezing. Preliminary work from our lab shows that prolonged depolarization alone can damage muscle, suggesting that cold-induced depolarization may be a key injury mechanism. To investigate this, I examined the femur**,** specifically the depressor and levator muscles (Figure 1), which control leg extension and flexion during walking. These muscles are structurally organized into repeating sarcomeres, each composed of Z-discs that anchor actin filaments and M-lines that organize myosin thick filaments (Figure 2). Together, these elements generate the contractile forces required for coordinated locomotion.

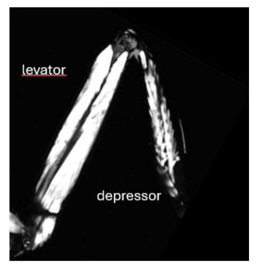

Figure 1. The *Drosophila* femur muscles. Fluorescence microscopy image of the two femur muscle types, the levator and depressor.[1]

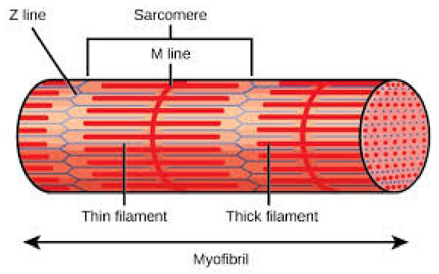

Figure 2. Schematic drawing of muscle organization.[2]

Objectives

1. Determing whether freezing damages muscles in D. melanogaster.
2. Test whether muscle structure recovers after freezing.
3. Assess how age and sex influence damage and recovery.

Methods

To visualize the muscle structure, we have used flies of Zasp52^{mCherry}; Obscurin^{GFP} genotype to visualize the Z disc and M line under a fluorescence microscope. Flies are aged into four cohorts (weeks 1-4) and separated by sex. Preliminary testing of walking speed assays and confocal microscopy of leg muscles is conducted to assess baseline walking speeds and muscle structure.

Flies were then subjected to a two-phase cold-hardening and freezing protocol (3°C for 3.5h, -4°C for 6h) (Figure 3), followed by seven days of recovery during which locomotor performance and muscle morphology were repeatedly assessed. Cold-hardening (CH) is a short-term acclimatory response that enhances survival following acute cold exposure[3]. This process allows flies to withstand freezing long enough to examine the consequences of that exposure. Immediately after freezing and for seven days following exposure, walking assays are conducted as well as confocal microscopy to assess damage and subsequent recovery of the depressor and levator muscles within the *Drosophila* femur.

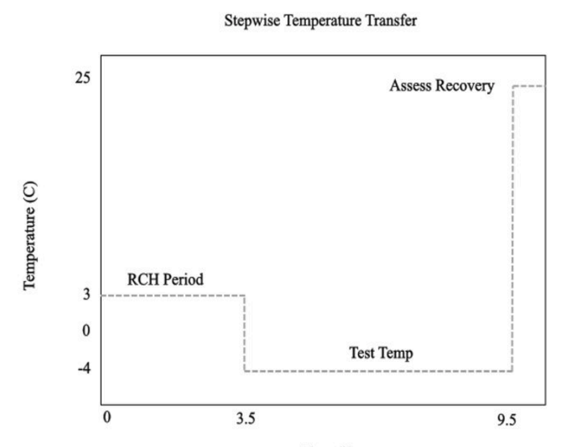

Figure 3. Stepwise temperature transfer protocol used to induce rapid cold-hardening (RCH). Flies were first held at 3 °C for 3.5 hours (RCH period), allowing physiological acclimation to mild cold. Temperature was then decreased to –4 °C for 6 hours to impose the damaging cold exposure. Following this test period, flies were returned to 25 °C to assess recovery.

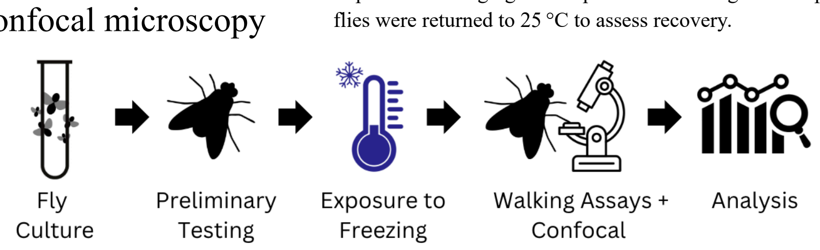

Figure 4. Experimental set up. Flies are cultured to produce desired genotype, preliminary testing of walking assays and confocal microscopy conducted, flies are exposed to freezing conditions using CH protocol, walking assays and confocal microscopy conducted immediately after and for 7 days following freezing, data is analyzed.

Results

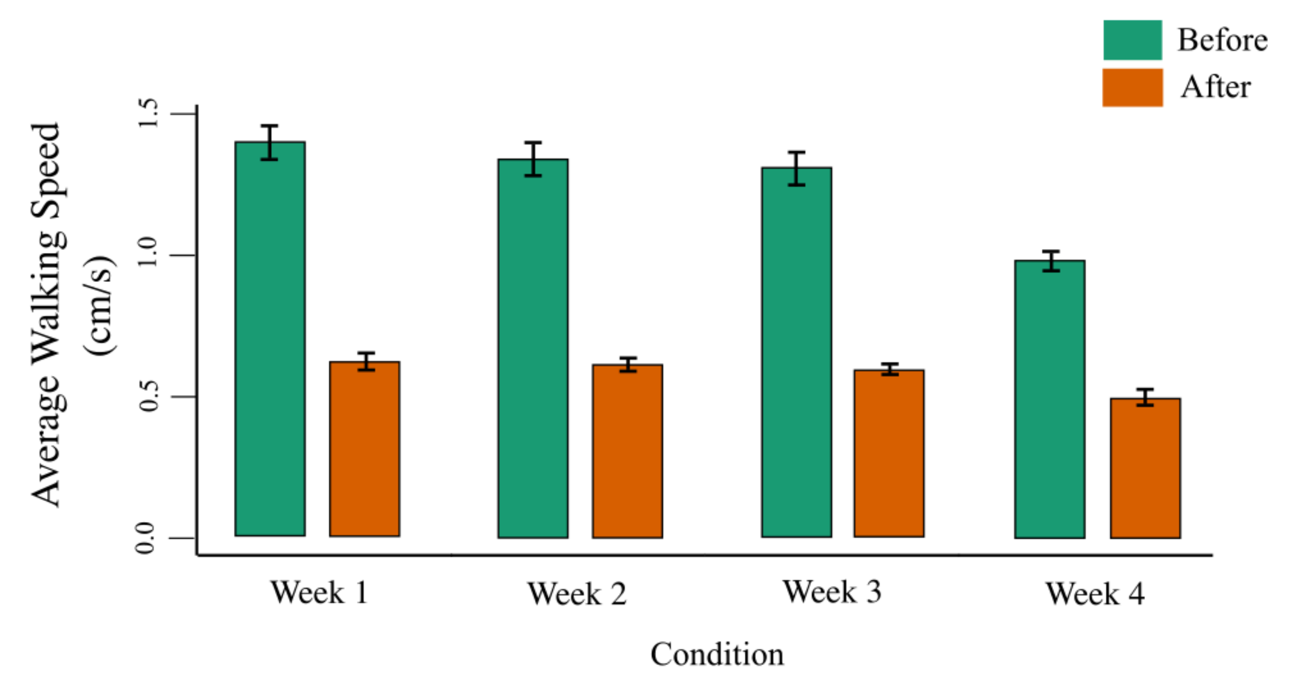

Figure 5. Walking speed before and immediately after freezing across four weekly trials. Each pair of bars represents the average walking speed (cm/s) of *Drosophila* measured prior to freezing ("Before," green) and immediately following freezing exposure ("After," orange) for Weeks 1 through 4. Across all time points, freezing consistently reduced locomotor performance, indicating acute muscle damage. Error bars represent standard error of the mean. This figure demonstrates the reproducibility of freezing-induced impairment and supports the use of walking speed as a functional readout of muscle integrity.

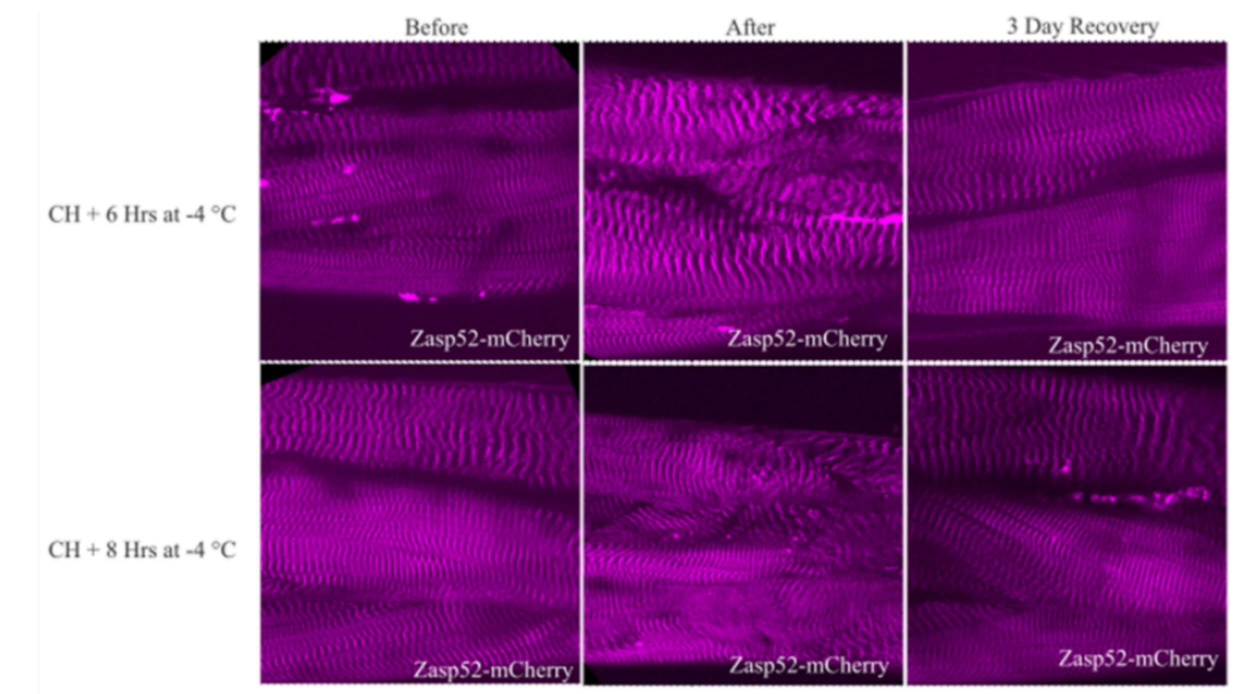

Figure 6. Muscle integrity before, immediately after, and following recovery from cold exposure. Representative fluorescent micrographs of *Drosophila* muscle labeled with Zasp52^{mCherry}, highlighting Z-disc organization under two cold exposure conditions: CH + 6 hrs at –4 °C (top row) and CH + 8 hrs at –4 °C (bottom row). Columns show muscle structure before freezing (left), immediately after freezing (middle), and following a 3-day recovery period (right). Cold exposure disrupted Z-disc alignment and muscle fiber organization, with similar structural damage observed after 8 hours compared to 6 hours. Partial restoration of muscle integrity was evident after 3 days of recovery, though residual disorganization persisted.

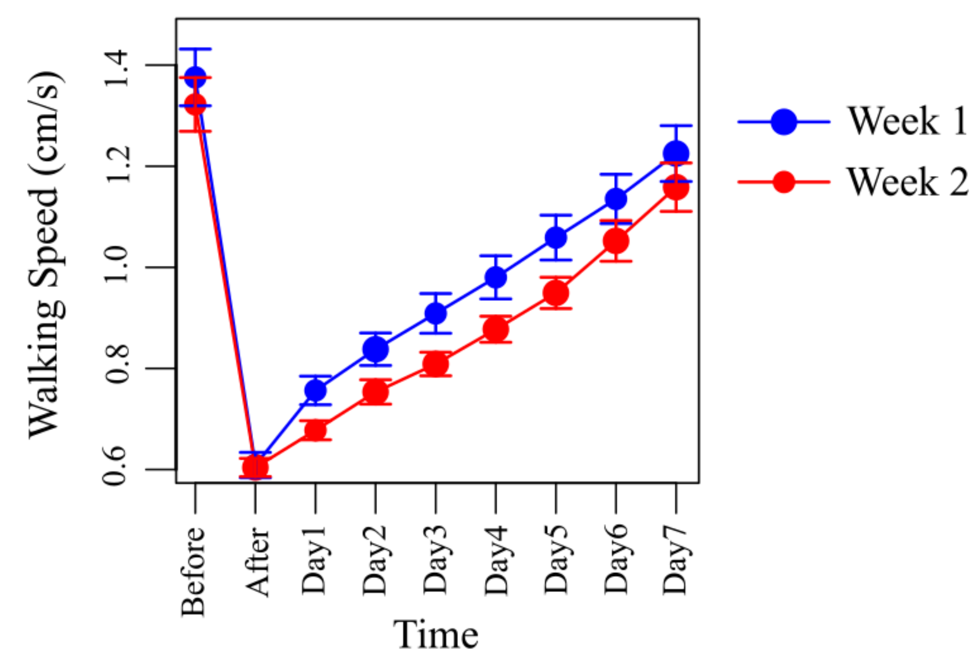

Figure 7. Walking speed recovery trajectories following freezing in week-1 and week-2 flies. Average locomotor performance (cm/s) was measured before freezing, immediately after freezing, and daily for seven days of recovery. Both age groups showed a sharp decline in walking speed immediately after freezing, consistent with acute muscle damage. Gradual improvement was observed over the recovery period, with walking speed increasing from Day 1 through Day 7. Week-2 flies (red line) exhibited slower recovery compared to week-1 flies (blue line), suggesting an age-dependent effect on muscle repair capacity. Error bars represent standard error of the mean.

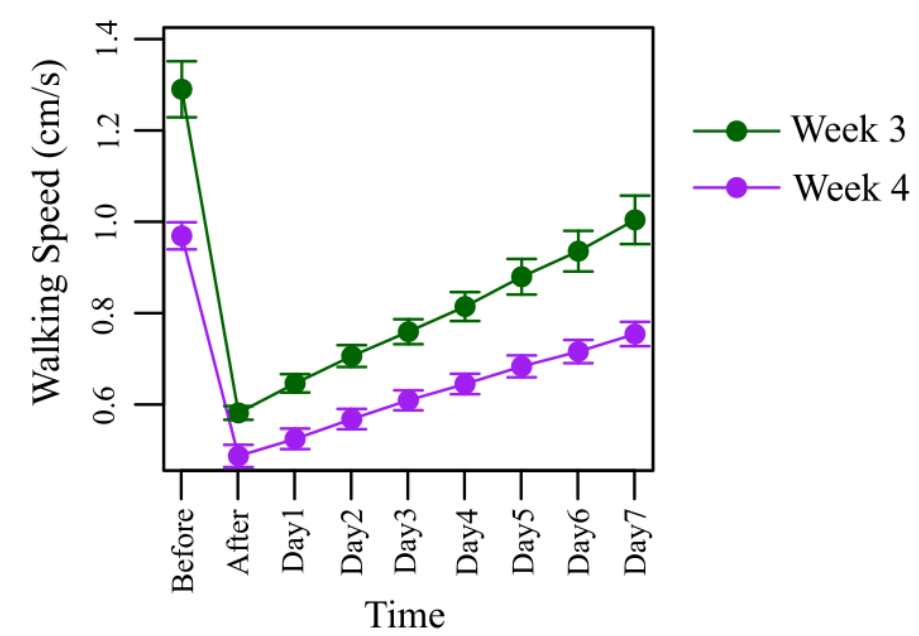

Figure 8. Walking speed recovery trajectories following freezing in week-3 and week-4 flies. Average locomotor performance (cm/s) was measured before freezing, immediately after freezing, and daily for seven days of recovery. Both age groups showed a sharp decline in walking speed immediately after freezing, consistent with acute muscle damage. Week-3 flies (green line) exhibited a faster recovery, regaining higher walking speeds by Day 7, whereas week-4 flies (purple line) recovered more slowly and incompletely. Error bars represent standard error of the mean. These results highlight an age-dependent decline in muscle repair capacity.

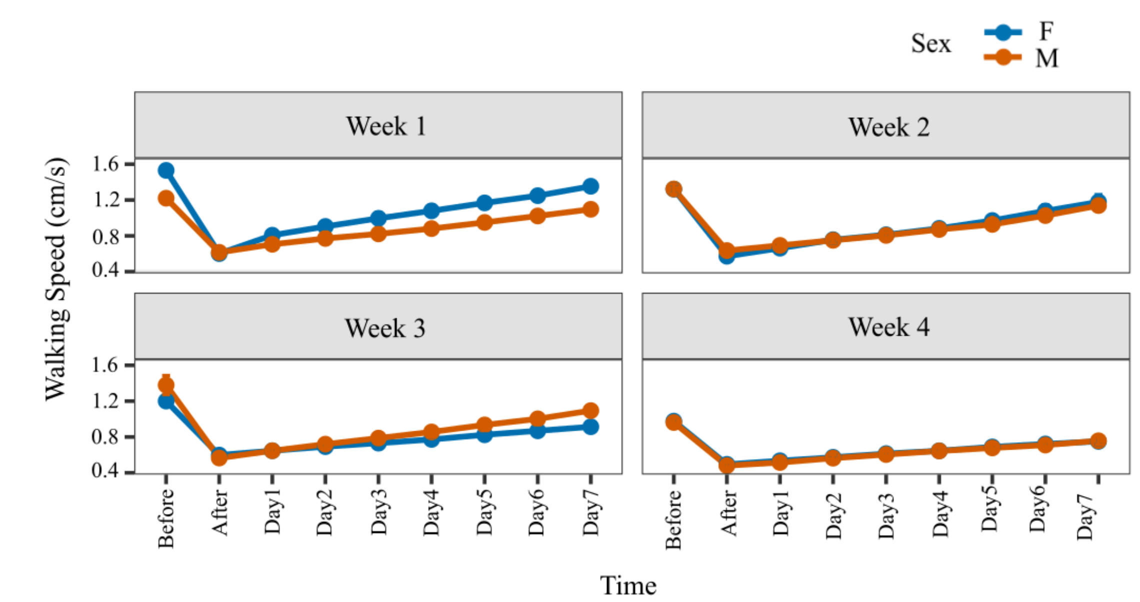

Figure 9. Sex-based walking speed recovery trajectories following freezing across four age groups. Line graphs show average walking speed (cm/s) for female (blue) and male (orange) *Drosophila* measured before freezing, immediately after freezing, and daily for seven days of recovery in Week 1, Week 2, Week 3, and Week 4 flies. In all age groups, walking speed declined sharply after freezing, followed by gradual recovery. Male and female trajectories were largely similar across time points, with minor sex-based variation in recovery rates. These data suggest that sex may play a minimal role in influencing recovery dynamics, while age may play a more prominent role.

Summary

1. Freezing induces measurable muscle damage and muscle fibres show clear evidence of repair.
2. Age strongly influences vulnerability and recovery.
3. Sex likely plays a minimal role.

Acknowledgements

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References

[1] Baek and Mann. (2009). Lineage and birth date specify motor neuron targeting and dendritic architecture in adult Drosophila. DOI: 10.1523/JNEUROSCI.1585-09.2009
[2] TeachMePhysiology (2025). Skeletal Muscle. https://teachmephysiology.com/histology/tissue-structure/muscle-histology/skeletal-muscle/
[3] Cjaka and Lee. (1988). Cold shock and rapid cold hardening in *Drosophila melanogaster*.